New developments in vaccines against African swine fever

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International Veterinary Vaccinology Network Webinar
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Spread of African Swine Fever Virus

Dixon et al., Antiviral Research 2019
African Swine Fever Status (2016-2020)
African Swine Fever in Africa

- Large DNA virus, Asfaviridae family
- Approximately 160 genes, number depending on isolate.
- 2 genotypes present in China, one in Europe.
- ASFV present in about 26 African countries.
- All 24 genotypes are present in Africa.
- There is a wildlife reservoir: warthogs and bush pigs.
- Wild boars are susceptible.
- Soft ticks of the genus Ornithodores are involved in transmission of ASFV.

Neighbor-Joining tree depicting the p72 gene relationships and geographical distribution of the major ASFV genotypes

Genotype I

Genotype II, III, IV, V, VI, VII, XIX, XX, XIII

Genotype VIII, XI, XII, XIII, XV, XVI, XIV

Genotype IX, X

Contribution: Livio Heath (ARC-OVI)
TOWARDS A LIVE ATTENUATED VACCINE FOR AFRICAN SWINE FEVER
Vaccine Candidates

Development of a Highly Effective African Swine Fever Virus Vaccine by Deletion of the I177L Gene Results in Sterile Immunity against the Current Epidemic Eurasia Strain


Deletion of the African Swine Fever Virus Gene DP148R Does Not Reduce Virus Replication in Culture but Reduces Virus Virulence in Pigs and Induces High Levels of Protection against Challenge


The Pirbright Institute, Pirbright, Woking, Surrey, United Kingdom

First Oral Vaccination of Eurasian Wild Boar Against African Swine Fever Virus Genotype II

Jose A Barasona, Carmina Gallardo, Estefania Cadenas-Fernandez, Cristina Jurado, Belen Rivera, Antonio Rodriguez-Bertos, Marisa Arias, Jose M Sanchez-Vizcaino

African Swine Fever Virus Bearing an I226R Gene Deletion Elicits Robust Immunity in Pigs to African Swine Fever

Yanyan Zhang, Junnan Ke, Jingyuan Zhang, Jinjun Yang, Huixian Yue, Xintao Zhou, Yu Qi, Fongnian Zhu, Faming Miao, Qian Li, Fei Zhang, Ying Wang, Xun Han, Lijuan Mu, Jinwei Yang, Shoufen Zeng, Teng Chen, Fengliang Hu

The International Livestock Research Institute, Nairobi, Kenya, and the CGIAR Research Program on Livestock Systems (RPLS)
Vaccine Candidates

• **Efficacy**
  - High level of protection, 100 % in many cases in various doses
  - Under optimal timing, 4 weeks post immunization
  - Duration of immunity ?

• **Safety**
  - Different dose studies for some vaccine candidates.
  - Very different clinical readout system, some use clinical score systems with many parameters (King 2011 and Galindo-Cardiel 2013), others use single parameters, e.g., fever.

• **Route of immunization**
  - Initially: intramuscularly
  - Orally route became interesting because of wild boar
  - Less viremia using orally route

Absence of Long-Term Protection in Domestic Pigs Immunized with Attenuated African Swine Fever Virus Isolate OURT88/3 or BeninaΔMFG Correlates with Increased Levels of Regulatory T Cells and Interleukin-10

Pedro J Sánchez-Cordón 1, Tamara Jabbar 2, Dave Chapman 2, Linda K Dixon 2, 3, Maria Montoya 2, 3

ILRI INTERNATIONAL LIVESTOCK INSTITUTE
CGIAR
ILRI ASFV Vaccine Activities

• Live vaccine (CRISPR/Cas9 deletions) and synthetic approach
  • Deletion of genes for attenuation
  • Testing in established animal model

• Subunit vaccine – activities
  • Screening of antigens
  • Viral vectors as delivery
Isolated Virus

- Kenya 1033 (genotype IX) isolated by ILRI and DVS Kenya.
- Genotype IX and X are especially circulation in Eastern Africa.
- Isolated from a zone with outbreaks.
- Used as the challenging virus in the animal model
- Used as backbone for deletion of genes to generate attenuated viruses.

Gallardo C et al. A.J. Biotech 2011
Onzere C. et al. Virus Genes 2018
ASFV Kenya 1033 – Virus Batch for Challenge

- This virus is very similar to the other genotype IX and X viruses.
- Animal model was set up. Different doses were tested.

5 animal per group, intramuscular injection.

Scoring system: Galindo-Cardiel 2013
Genomic Stability and Production Cell Lines

Problems with instability of genomes in cell lines

- ZMAC – pig macrophage cell line
- MA-104 cell line (Green monkey kidney epithelial cell line)

Progress on production cell lines

- A porcine macrophage cell line that supports high levels of replication of OURT88/3, an attenuated strain of African swine fever virus
- Identification of a Continuously Stable and Commercially Available Cell Line for the Identification of Infectious African Swine Fever Virus in Clinical Samples
Virulence of WSL Adapted WT-Virus

- WSL (from FLI) is a fetal wild boar lung cell line, not immortalized.
- ASFV Kenya 1033 was adapted to WSL (20+ passages)
- \(10^2\) TCID\(_{50}\) was chosen to test if the virus grown in WSL cells was still lethal

Challenge with wild type virus.
Open circles: WSL cell line grown virus, Solid squares: Macrophage grown virus

Scoring system: King et al. 2011
Titers of ASFV Ken-1033 in WSL

- Titers for Macrophage grown ASFV - 4 days
- Titers for WSL grown ASFV - 4 days

- MOI 0.1
- MOI 0.25
- MOI 1
- MOI 2.5
- MOI 5
CRISPR-Cas Editing of African Swine Fever Virus

Infect with WT-virus

Transfection with guide RNA and GFP donor DNA

Stable CAS transfected cell line (WSL)

Check for GFP insert with PCR over junction

Cloning of cells with fluorescent virus

➢ Directly modification on replicating virus inside cells

Constructed 7-10 different viruses
Synthetic Construction of African Swine Fever Virus

➢ Capacity to efficiently perform genome-wide changes in the virus genome in a combinatorial manner to understand virus biology.

➢ Capacity to produce clinically-relevant viruses without extensive passaging in tissue culture.

➢ Streamlines process to generate various designer vaccine candidates and oncolytic viruses.
First Viruses: Experimental Setup

<table>
<thead>
<tr>
<th>Immunisation (1 injection)</th>
<th>Challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td>$10^4$ ASF1033_ΔCD2v</td>
<td>$10^2$ ASF1033 (8x)</td>
</tr>
<tr>
<td>$10^4$ ASF1033_ΔCD2vΔA238L</td>
<td>$10^2$ ASF1033 (8x)</td>
</tr>
<tr>
<td>PBS</td>
<td>$10^2$ ASF1033 (8x)</td>
</tr>
</tbody>
</table>

- **CD2v**: Immunomodulatory molecule promoting apoptosis of lymphocytes.
- **A238L**: Mimic NFκB subunit, inhibits NFκB activity, which is crucial in the pro-inflammatory response.
Clinical Scores After Immunization

- ASF1033_ΔCD2v
- ASF1033_ΔCD2vΔA238L
- PBS

Weight gain and Post-immunisation graphs are also shown.
Clinical Scores After Challenge

- **ASF1033_ΔCD2v**
  - Clinical score vs. DPC
  - Graph showing the clinical score over time for ASF1033_ΔCD2v.

- **ASF1033_ΔCD2vΔA238L**
  - Clinical score vs. DPC
  - Graph showing the clinical score over time for ASF1033_ΔCD2vΔA238L.

- **PBS**
  - Clinical score vs. DPC
  - Graph showing the clinical score over time for PBS.

**Challenge**

Graphs comparing clinical scores after challenge for different conditions (ASF1033_ΔCD2v, ASF1033_ΔCD2vΔA238L, PBS) over time (DPC).
Survival Plot

Survival proportions

- ASF1033_ΔCD2v
- ASF1033_ΔCD2vΔA238L
- PBS

IFNγ-ELISpot D28

- green circle: ASF1033_ΔCD2v
- red square: ASF1033_ΔCD2vΔA238L
- blue triangle: PBS
Conclusion

• \( \Delta \text{CD2v} \) is more efficient than the double knockout but less attenuated. 87.5% protection versus 50%.

• \( \Delta \text{A238L} \) seems to add to the attenuation, but with a loss in ability to protect.
Second viruses: Experimental Setup

<table>
<thead>
<tr>
<th>Immunisation (1 injection)</th>
<th>Challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td>$10^3$ ASF1033_∆X (7x)</td>
<td>$10^2$ ASF1033_∆X (6x)</td>
</tr>
<tr>
<td>$10^3$ ASF1033_∆Y (7x)</td>
<td>$10^2$ ASF1033_∆Y (6x)</td>
</tr>
<tr>
<td>PBS (7x)</td>
<td>$10^2$ ASF1033 (6x)</td>
</tr>
</tbody>
</table>

- **Quarantine**
  - Day -21
  - Day 0

- **Clinical scoring**
  - Day 28
  - Day 51
New Gene-Deleted Viruses

All groups

Immunization

Challenge

Clinical scores (mean)

Days post infection

Group 1

Group 2

Group 3

IFNγ-ELISpot D28

SFU/10^6 PBM

G group 1

G group 2

G group 3

Remaining data:

Viremia data

Antibody titers

PM data
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ΔCD2v virus / WT-virus

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